A

Please type a plus sign (+) inside this box → [+]

PTO/SB/05 (12/97)
Approved for use through 09/30/00. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	2509-980383
Total Pages	27
First Named Inventor or Application Identifier	Paul L. Kornblith
Express Mail Label No.	EL037027165US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] Fee Transmittal Form *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [Total Pages 14] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [ ] Drawing(s) *(35 USC 113)* [Total Sheets]
4. Oath or Declaration [Total Pages 2]
   a. [ ] Newly executed (orginal or copy)
   b. [X] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed) [Note Box 5 below]*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
5. [X] Incorporation By Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [X] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. [ ] Small Entity Statement(s) [X] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [X] Other: Assignment filed in prior application

17. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information:*
[X] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: 08 / 679,056

18. CORRESPONDENCE ADDRESS

[ ] Customer Number or Bar Code Label *(Insert Customer No. or Attach bar code label here)* or [X] Correspondence address below

NAME	Barbara E. Johnson				
ADDRESS	700 Koppers Building 436 Seventh Avenue				
CITY	Pittsburgh	STATE	PA	ZIP CODE	15219-1818
COUNTRY	U.S.A.	TELEPHONE	412-471-8815	FAX	412-471-4094

Attorney's Docket No. 2509-980383

PATENT APPLICATION TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

Transmitted herewith for filing is the patent application of:
Paul L. Kornblith
Entitled: Method for Preparing Cell Cultures from Biological Specimens for Chemotherapeutic and Other Assays

Enclosed are:

_____ _____sheet/s of drawing/s
_____ An Assignment of the invention to:_____
_____
_____ Declaration
_____ Small Entity Statement under 37 CFR 1.9 and 1.27

CLAIMS AS FILED

	No. Filed	No. Extra		Small Entity Rate	Non-Small Entity Rate	Charge
Total Claims	12	-20 = 0	X	$ 11.00	$ 22.00	$ 0
Indep. Claims	1	- 3 = 0	X	$ 41.00	$ 82.00	$ 0
Multiple Dependent Claim/s				$135.00	$270.00	$ 0
Basic Fee +				$395.00	$790.00	$ 395
				Total of above Charges		$ 395
				Total Fee		$ 395

A check/s in the amount/s of $ 395 is/are enclosed to cover the filing fee and _____.

The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 CFR 1.16 or patent application processing fees under 37 CFR 1.17 associated with this communication to Deposit Account No. 23-0650. Please refund any overpayment in the form of a check.

An original and two copies of this sheet are enclosed.

March 17, 1998
Date

By _____
Barbara E. Johnson
Attorney for Applicant
Registration No. 31,198
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219-1818
Telephone: 412-471-8815
Facsimile: 412-471-4094

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	ATTORNEY'S DOCKET NUMBER
Paul L. Kornblith	**2509-980383**

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, DC 20231

EXPRESS MAIL CERTIFICATE

"Express Mail" label number EL037027165US

Date of Deposit March 17, 1998

I hereby certify that the following attached paper or fee

Preliminary Amendment (4 pp.)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Kara A. Toward
(Typed name of person mailing paper or fee)

Kara A. Toward
(Signature of person mailing paper or fee)

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**METHOD FOR PREPARING CELL CULTURES FROM BIOLOGICAL**
Paul L. Kornblith	:	**SPECIMENS FOR CHEMOTHERAPEUTIC AND**
Filed March 17, 1998	:	**OTHER ASSAYS**

Pittsburgh, Pennsylvania
March 17, 1998

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Please delete the title, "PRECISE EFFICACY ASSAY METHODS FOR ACTIVE AGENTS INCLUDING CHEMOTHERAPEUTIC AGENTS" and insert therefor --METHOD FOR PREPARING CELL CULTURES FROM BIOLOGICAL SPECIMENS FOR CHEMOTHERAPEUTIC AND OTHER ASSAYS--.

Page 1, after the title, insert the following:

--Related Application

This application is a continuation of U.S. Application Serial No. 08/679,056, filed July 12, 1996.--

Please replace the Abstract of the Disclosure on page 14 with the new Abstract of the Disclosure provided herein on substitute page 14.

IN THE CLAIMS:

Please cancel claims 1-12 and substitute therefor new claims 13-19 as follows:

--13. A method for preparing a biopsy sample of tissue containing malignant cells for chemosensitivity testing, comprising:

(a) obtaining a tumor specimen;

(b) mechanically separating said specimen into cohesive multicellular particulates;

(c) growing a tissue culture monolayer from said cohesive multicellular particulates;

(d) inoculating cells from said monolayer into a plurality of segregated sites;

(e) treating said plurality of sites with at least one agent; and

(f) assessing chemosensitivity of the cells in said plurality of sites.

14. The method according to claim 13 wherein said plurality of segregated sites comprise a plate containing a plurality of wells therein.

15. The method according to claim 14 wherein said plurality of wells is treated over a length of time adequate to permit assessment of both initial cytotoxic effect and longer-term inhibitory effect of at least one of said plurality of active agents.

16. The method according to claim 15 wherein step (d) is accomplished by a multiple well pipetting device.

17. The method according to claim 16 wherein the cells in step (d) are further suspended in medium prior to inoculation into said plate containing a plurality of wells therein.

18. The method according to claim 17 wherein said agent is at least one of the agents selected from the group consisting of a radiation therapy agent, a radiation therapy sensitizing agent and a radiation therapy desensitizing agent.

19. The method according to claim 17 wherein said agent is an immunotherapeutic agent.--

REMARKS

As discussed among Dr. Kornblith, Examiner Gitomer and the undersigned in a telephone interview of record in the parent application hereto, Applicants continue to believe that the invention is embraced by the recitation "cohesive multicellular particulates" without the limitation "having a size of about 1 mm^3." It is the purpose of this continuing application to address this issue directly.

Examination and allowance of claims 13-19 are respectfully requested.

Respectfully submitted,

WEBB ZIESENHEIM BRUENING LOGSDON
ORKIN & HANSON, P.C.

By ______________________
Barbara E. Johnson
Reg. No. 31,198
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219-1818
Attorney for Applicant
Telephone: (412) 471-8815
Facsimile: (412) 471-4094

Abstract of the Disclosure

An improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent for the particular patient. The system includes the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most promising agent and concentration for treatment of a particular patient can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a given anti-cancer agent are investigated.

PRECISE EFFICACY ASSAY METHODS FOR ACTIVE AGENTS INCLUDING CHEMOTHERAPEUTIC AGENTS

Field of the Invention

The invention relates to screening and testing of active agents, including chemotherapeutic agents, to predict potential efficacy in individual patients in whom treatment with such agents is indicated.

Introduction

All active agents including chemotherapeutic active agents are subjected to rigorous testing as to efficacy and safety prior to approval for medical use in the United States. Methods of assessing efficacy have included elaborate investigations of large populations in double blind studies as to a given treatment method and/or active agent, with concommitant statistical interpretation of the resulting data, but these conclusions are inevitably generalized as to patient populations taken as a whole. In many pharmaceutical disciplines and particularly in the area of chemotherapy, however, the results of individual patient therapy may not comport with generalized data--to the detriment of the individual patient. The need has been long recognized for a method of assessing the therapeutic potential of active agents, including but not limited to chemotherapeutic agents, for their efficacy as to a given individual patient, prior to the treatment of that patient.

Prior art assays already exist which expose malignant tissue of various types to a plurality of active agents, for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity," Anticancer Research, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell-lung cancers was investigated in in vivo grafts, in in vitro primocultures and in commercially available long-term cancer cell lines. The increase in chemosensitivity was documented and

correlated with morphological changes in the cells in question. Sometimes animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see for example Arnold, J.T., "Evaluation of chemopreventive agents in different mechanistic classes using a rat tracheal epithelial cell culture transformation assay," Cancer Res., vol. 55, no. 3, pp. 537-543 (1995).

When actual patient cells are used to form in vitro assays focussed on individual patients, in typical prior art processes the cells are harvested (biopsied) and trypsinized (connective tissue digested with the enzyme trypsin) to yield a cell suspension suitable for conversion to the desired tissue culture form. The in vitro tissue culture cell collections which result from these techniques are generally plagued by their inability accurately to imitate the chemosensitivity of the original tumor or other cell biopsy. Standard cloning and tissue culture techniques are moreover excessively complicated and expensive for use in a patient-by-patient assay setting. A need thus remains for a technique of tissue culture preparation which provides cell cultures, for drug screening purposes, in which after simple preparation the cell cultures react in a manner equivalent to their in vivo reactivity, to enable drug or chemotherapeutic agent screening as to a particular patient for whom such screening is indicated.

Summary of the Invention

In order to meet this need, the present invention is an improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment for the cultured cells obtained from the patient. Specific method innovations such as tissue sample preparation

techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. Growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most efficacious agent can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a given anti-cancer agent are investigated.

Detailed Description of the Invention

The present invention is a system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One

particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates (explants) of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. Cell growth, and sequence and timing of drug addition, are monitored and optimized.

An important application of the present invention is the screening of chemotherapeutic agents and other antineoplastic therapies against tissue culture preparations of malignant cells from the patients from whom malignant samples are biopsied. Related anti-cancer therapies which can be screened using the inventive system are both radiation therapy and agents which enhance the cytotoxicity of radiation, as well as immunotherapeutic anti-cancer agents. Screening processes for treatments or therapeutic agents for nonmalignant syndromes are also embraced within this invention, however, and include without limitation agents which combat hyperproliferative syndromes, such as psoriasis, or wound healing agents. Nor is the present efficacy assay limited only to the screening of active agents which speed up (healing) or slow down (anti-cancer, anti-hyperproliferative) cell growth because agents intended to enhance or to subdue intracellular biochemical functions may be tested in the present tissue culture system also. For example, the formation or blocking of enzymes, neurotransmitters and other biochemicals may be screened with the present assay methods prior to treatment of the patient.

When the patient is to be treated for the presence of tumor, in the preferred embodiment of the present invention a tumor biopsy of >100 mg of non-necrotic, non-contaminated tissue is harvested from the patient by any suitable biopsy or surgical procedure known in the art. Biopsy sample preparation generally proceeds as follows under a Laminar Flow Hood which should be turned on at least 20 minutes before use. Reagent grade ethanol

is used to wipe down the surface of the hood prior to beginning the sample preparation. The tumor is then removed, under sterile conditions, from the shipping container and is minced with sterile scissors. If the specimen arrives already minced, the individual tumor pieces should be divided into four groups. Using sterile forceps, each undivided tissue quarter is then placed in 3 ml sterile growth medium (Standard F-10 medium containing 17% calf serum and a standard amount of Penicillin and Streptomycin) and systematically minced by using two sterile scalpels in a scissor-like motion, or mechanically equivalent manual or automated opposing incisor blades. This cross-cutting motion is important because the technique creates smooth cut edges on the resulting tumor multicellular particulates. Preferably but not necessarily, the tumor particulates each measure 1 mm^3. After each tumor quarter has been minced, the particles are plated in culture flasks using sterile pasteur pipettes (9 explants per T-25 or 20 particulates per T-75 flask). Each flask is then labelled with the patient's code, the date of explantation and any other distinguishing data. The explants should be evenly distributed across the bottom surface of the flask, with initial inverted incubation in a 37° C. incubator for 5-10 minutes, followed by addition of about 5-10 ml sterile growth medium and further incubation in the normal, non-inverted position. Flasks are placed in a 35° C, non-CO_2 incubator. Flasks should be checked daily for growth and contamination. Over a period of a few weeks, with weekly removal and replacement of 5 ml of growth medium, the explants will foster growth of cells into a monolayer. With respect to the culturing of malignant cells, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells

themselves is facilitated versus the overgrowth of fibroblasts (or other unwanted cells) which tends to occur when suspended tumor cells are grown in culture.

The use of the above procedure to form a cell monolayer culture maximizes the growth of malignant cells from the tissue sample, and thus optimizes ensuing tissue culture assay of chemotherapeutic action of various agents to be tested. Enhanced growth of actual malignant cells is only one aspect of the present invention, however; another important feature is the growth rate monitoring system used to oversee growth of the monolayer once formed. Once a primary culture and its derived secondary monolayer tissue culture has been initiated, the growth of the cells is monitored to ascertain the time to initiate the chemotherapy assay and to determine the growth rate of the cultured cells.

Monitoring of the growth of cells is conducted by counting the cells in the monolayer on a periodic basis, without killing or staining the cells and without removing any cells from the culture flask. The counting may be done visually or by automated methods, either with or without the use of estimating techniques known in the art (counting in a representative area of a grid multiplied by number of grid areas, for example). Data from periodic counting is then used to determine growth rates which may or may not be considered parallel to growth rates of the same cells <u>in vivo</u> in the patient. If growth rate cycles can be documented, for example, then dosing of certain active agents can be customized for the patient. The same growth rate can be used to evaluate radiation treatment periodicity, as well. It should be noted that with the growth rate determinations conducted while the monolayers grow in their flasks, the present method requires no hemocytometry, flow cytometry or use of microscope slides and staining, with all their concommitant labor and cost.

Protocols for monolayer growth rate generally use a phase-contrast inverted microscope to examine culture flasks incubated in a 37° C. (5% CO_2) incubator. When the flask is placed under the phase-contrast inverted microscope, ten fields (areas on a grid inherent to the flask) are examined using the 10x objective, with the proviso that the ten fields should be non-contiguous, or significantly removed from one another, so that the ten fields are a representative sampling of the whole flask. Percentage cell occupancy for each field examined is noted, and averaging of these percentages then provides an estimate of overall percent confluency in the cell culture. When patient samples have been divided between two or among three or more flasks, an average cell count for the total patient sample should be calculated. The calculated average percent confluency should be entered into a process log to enable compilation of data--and plotting of growth curves--over time. Monolayer cultures may be photographed to document cell morphology and culture growth patterns. The applicable formula is:

$$\text{Percent confluency} = \frac{\text{estimate of the area occupied by cells}}{\text{total area in an observed field}}$$

As an example, therefore, if the estimate of area occupied by the cells is 30% and the total area of the field is 100%, percent confluency is 30/100, or 30.

Adaptation of the above protocol for non-tumor cells is straightforward and generally constitutes an equivalent procedure.

Active agent screening using the cultured cells does not proceed in the initial incubation flask, but generally proceeds using plates such as microtiter plates. The performance of the chemosensitivity assay used for screening purposes depends on the ability to deliver a reproducible cell number to each row in a plate and/or a series of plates, as well as the ability to achieve an even distribution of cells throughout a given well. The

following procedure assures that cells are reproducibly transferred from flask to microtiter plates, and cells are evenly distributed across the surface of each well.

The first step in preparing the microtiter plates is, of course, preparing and monitoring the monolayer as described above. The following protocol is exemplary and susceptible of variation as will be apparent to one skilled in the art. Cells are removed from the culture flask and a cell pellet is prepared by centrifugation. The cell pellet derived from the monolayer is then suspended in 5 ml of the growth medium and mixed in a conical tube with a vortex for 6 to 10 seconds. The tube is then rocked back and forth 10 times. A 36 μl droplet from the center of the conical tube is pipetted onto one well of a 96 well plate. A fresh pipette is then used to pipette a 36 μl aliquot of trypan blue solution, which is added to the same well, and the two droplets are mixed with repeated pipette aspiration. The resulting admixture is then divided between two hemocytometer chambers for examination using a standard light microscope. Cells are counted in two out of four hemocytometer quadrants, under 10x magnification. Only those cells which have not taken up the trypan blue dye are counted. This process is repeated for the second counting chamber. An average cell count per chamber is thus determined. Using means known in the art, the quadrant count values are checked, logged, multiplied by 10^4 to give cells/ml, and the total amount of fluid (growth medium) necessary to suspend remaining cell aliquots is calculated accordingly.

After the desired concentration of cells in medium has been determined, additional cell aliquots from the monolayer are suspended in growth medium via vortex and rocking and loaded into a Terasaki dispenser known in the art. Aliquots of the prepared cell suspension are delivered into the microtiter plates using Terasaki dispenser techniques known in the art. A plurality of plates may be prepared from a single cell suspension as

needed. Plates are then wrapped in sterile wet cotton gauze and incubated in an incubator box by means known in the art.

After the microtiter plates have been prepared, exposure of the cells therein to active agent is conducted according to the following exemplary protocol. During this portion of the inventive assay, the appropriate amount of specific active agent is tranferred into the microtiter plates prepared as described above. A general protocol, which may be adapted, follows. Each microtiter plate is unwrapped from its wet cotton gauze sponge and microscopically examined for cell adhesion. Control solution is dispensed into delineated rows of wells within the grid in the microtiter plate, and appropriate aliquots of active agent to be tested are added to the remaining wells in the remaining rows. Ordinarily, sequentially increasing concentrations of the active agent being tested are administered into progressively higher numbered rows in the plate. The plates are then rewrapped in their gauze and incubated in an incubator box at 37° C. under 5% CO_2. After a predefined exposure time, the plates are unwrapped, blotted with sterile gauze to remove the agent, washed with Hank's Balance Salt Solution, flooded with growth medium, and replaced in the incubator in an incubator box for a predefined time period, after which the plates may be fixed and stained for evaluation.

Fixing and staining may be conducted according to a number of suitable procedures; the following is representative. After removal of the plates from the incubator box, culture medium is poured off and the plates are flooded with Hank's Balance Salt Solution. After repeated flooding (with agitation each time) the plates are then flooded with reagent grade ethanol for 2-5 minutes. The ethanol is then poured off. Staining is accomplished with approximately 5 ml of Giemsa Stain per plate, although volume is not critical and flooding is the goal. Giemsa stain should be left in place 5 min. ± 30 seconds as timing

influences staining intensity. The Giemsa stain is then poured off and the plates are dipped 3 times cold tap water in a beaker. The plates are then inverted, shaken vigorously, and air dried overnight (with plate lids off) on a rack on a laboratory bench. Cells per well are then counted manually or by automated and/or computerized means, to derive data regarding chemosensitivity of cells at various concentrations of exposure. One particularly useful computer operating environment for counting cells is the commercially available OPTIMATE compiler, which is designed to permit an optical counting function well suited to computerized cell counting procedures and subsequent calculations.

The above procedures do not change appreciably when cell growth promoters are assayed rather than cell arresting agents such as chemotherapeutic agents. The present assay allows cell death or cell growth to be monitored with equal ease. In any case, optimization of use of the present system will involve the comparative testing of a variety of candidate active agents, for selection of the best candidate for patient treatment based upon the in vitro results. One particularly advantageous embodiment of the above described invention comprises a two-stage assay for cytotoxicity followed by evaluation of longer-term inhibitory effect. Chemotherapeutic agents may thus be evaluated separately for both their direct chemotherapeutic effect as well as for their longer duration efficacy.

Identification of one or more active agents or chemotherapeutic agents is peripheral to the present invention, which is intended for the efficacy screening of any or all of them as to a given patient. Literally any active agent may be screened according to the present invention; listing exemplary active agents is thus omitted here.

The essence of the invention thus includes the important feature of the simplicity of the present system--cohesive multicellular particulates of the patient tissue to be tested are used to form cell monolayers; growth of those monolayers is monitored for accurate prediction of correlating growth of the same cells in vivo; and differing concentrations of a number of active agents may be tested for the purpose of determining not only the most appropriate agent but the most appropriate concentration of that agent for actual patient exposure (according to the calculated cell growth rates). It is also important to note, in the context of the invention, that the present system allows in vitro tests to be conducted in suspensions of tissue culture monolayers grown in nutrient medium under fast conditions (a matter of weeks), rather than with single cell progeny produced by dilution cloning over long periods of time. In some cases, the present invention is a two stage assay for both cytotoxicity and the longer-term growth inhibitory.

Although the present invention has been described with respect to specific materials and methods above, the invention is only to be considered limited insofar as is set forth in the accompanying claims.

I claim:

1. A method for assessing chemosensitivity of patient cells comprising the steps of:

a) harvesting a specimen of a patient's tissue, cells ascites, or effusion fluid;

b) separating said specimen into multicellular particulates;

c) growing a tissue culture monolayer from said cohesive multicellular particulates;

d) inoculating cells from said monolayer into a plurality of segregated sites; and

e) treating said plurality of sites with at least one active agent, followed by assessment of chemosensitivity of the cells in said site to at least one active agent.

2. The method according to claim 1 wherein step a) further comprises the step of

a) preparing a specimen which was harvested from a sample of patient tumor tissue;.

3. The method according to claim 1 wherein said plurality of segregated sites further comprises a plate containing a plurality of wells therein.

4. The method according to claim 1 wherein step e) further comprises the step of:

e) treating said plurality of sites with a plurality of active agents at varied concentrations, followed by assessment of optimal chemosensitivity with respect to a single active agent at a single concentration.

5. The method according to claim 1 wherein step e) further comprises the step of:

e) treating said plurality of sites with a plurality of active agents over a length of time adequate to permit assessment of both initial cytotoxic effect and longer-term inhibitory effect of at least one of said plurality of active agents.

6. The method according to claim 1 wherein the chemosensitivity assayed according to step e) is anti-cancer sensitivity.

7. The method according to claim 1 wherein step d) is accomplished using a Terasaki dispenser.

8. The method according to claim 1 wherein the cells in step d) are prepared in suspension prior to inoculation into a plurality of wells in a culture plate.

9. The method according to claim 1, wherein said active agent is a chemotherapeutic agent.

10. The method according to claim 1, wherein said active agent is a wound healing agent.

11. The method according to claim 1, wherein said active agent is a radiation therapy and/or a radiation therapy sensitizing or ameliorating agent.

12. The method according to claim 1, where said active agent is an immunotherapeutic agent.

Abstract of the Disclosure

An improved system for screening a multiple of candidate therapeutic or chemotherapeutic agents for efficacy as to a specific patient, in which a tissue sample from the patient is harvested, cultured and separately exposed to a plurality of treatments and/or therapeutic agents for the purpose of objectively identifying the best treatment or agent for the particular patient. Specific method innovations such as tissue sample preparation techniques render this method practically as well as theoretically useful. One particularly important tissue sample preparation technique is the initial preparation of cohesive multicellular particulates of the tissue sample, rather than enzymatically dissociated cell suspensions or preparations, for initial tissue culture monolayer preparation. With respect to the culturing of malignant cells, for example, it is believed (without any intention of being bound by the theory) that by maintaining the malignant cells within a multicellular particulate of the originating tissue, growth of the malignant cells themselves is facilitated versus the overgrowth of fibroblasts or other cells which tends to occur when suspended tumor cells are grown in culture. Practical monolayers of cells may thus be formed to enable meaningful screening of a plurality of treatments and/or agents. Growth of cells is monitored to ascertain the time to initiate the assay and to determine the growth rate of the cultured cells; sequence and timing of drug addition is also monitored and optimized. By subjecting uniform samples of cells to a wide variety of active agents (and concentrations thereof), the most promising agent and concentration for treatment of a particular patient can be determined. For assays concerning cancer treatment, a two-stage evaluation is contemplated in which both acute cytotoxic and longer term inhibitory effect of a given anti-cancer agent are investigated.

DECLARATION AND POWER OF ATTORNEY

Paul L. Kornblith, declares:

I am a citizen of the United States of America and a resident of the City of Pittsburgh, County of Allegheny, Commonwealth of Pennsylvania, whose post-office address is 907 Settler's Ridge Road, Pittsburgh, Pennsylvania 15238.

I believe myself to be the original, first and sole inventor of the improvement entitled "Precise Efficacy Assay Methods For Active Agents Including Chemotherapeutic Agents" which is described and claimed in U.S. Application Serial No. 08/679,056, filed July 12, 1996.

I have reviewed and understand the contents of the specification, including the claims.

I do not know and do not believe that the same was ever known or used in the United States before my invention thereof; or patented or described in any printed publication in any country before my invention or more than one year prior to this application; or in public use or on sale in the United States more than one year prior to this application.

Said invention has not been patented or been made the subject of an inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to this application.

I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

No application for patent or inventor's certificate thereon has been filed by me or my legal representatives or assigns in any country foreign to the United States.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

I hereby appoint Barbara E. Johnson, Registration No. 31,198; William H. Webb, Registration No. 13,467; David C. Bruening, Registration No. 20,742; William H. Logsdon, Registration No. 22,132; Russell D. Orkin, Registration No. 25,363; David C. Hanson, Registration No. 23,024; Richard L. Byrne, Registration No. 28,498; Frederick B. Ziesenheim, Registration No. 19,438; Kent E. Baldauf, Registration No. 25,826; Paul M. Reznick, Registration No. 33,059; John W.

McIlvaine, Registration No. 34,219; Michael I. Shamos, Registration No. 30,424; Blynn L. Shideler, Registration No. 35,034; Julie W. Meder, Registration No. 36,216; Lester N. Fortney, Registration No. 38,141; and Randall A. Notzen, Registration No. 36,882, whose post-office address is 700 Koppers Building, 436 Seventh Avenue, Pittsburgh, Pennsylvania 15219-1818, Telephone No. 412-471-8815, my attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, to amend the specification, to appeal in case of rejection, as they may deem advisable, to receive the patent when granted and generally to do all matters and things needful in the premises, as fully and to all intents and purposes as I could do.

All correspondence and telephone calls should be addressed to Barbara E. Johnson.

I hereby subscribe my name to the foregoing specification and claims, declaration and power of attorney this 25th day of Sept, 1996.

Inventor Paul L Kornblith
Paul L. Kornblith

Applicant or Patentee: Paul L. Kornblith Attorney's
Serial or Patent No.: 08/679,056 Docket No.: 2509-960878
Filed or Issued: July 12, 1996
For: Precise Efficacy Assay Methods For Active Agents Including Chemotherapeutic Agents

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c))--SMALL BUSINESS CONCERN

I hereby declare that I am

___ the owner of the small business concern identified below:
_X_ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Precision Therapeutics, Inc.
ADDRESS OF SMALL BUSINESS CONCERN 3636 Boulevard of the Allies, Pittsburgh, PA 15213

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Precise Efficacy Assay Methods For Active Agents Including Chemotherapeutic Agents by inventor(s) Paul L. Kornblith

described in

___ the specification filed herewith.
_X_ application serial no. 08/679,056, filed 7/12/96.
___ patent no. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
_____INDIVIDUAL _____SMALL BUSINESS CONCERN _____NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
_____INDIVIDUAL _____SMALL BUSINESS CONCERN _____NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Paul Kornblith

TITLE OF PERSON IF OTHER THAN OWNER President

ADDRESS OF PERSON SIGNING 3636 Boulevard of the Allies
Pittsburgh, PA 15213

SIGNATURE Paul Kornblith DATE 9/25/96.

United States Patent & Trademark Office

Office of Initial Patent Examination – Scanning Division

09040454.031798

Application deficiencies found during scanning:

1. Application papers are not suitable for scanning and are not in compliance with 37 CFR 1.52 because:
   - ☐ All sheets must be the same size and either A4 (21 cm x 29.7 cm) or 8-1/2" x 11" Pages ______________ do not meet these requirements.
   - ☐ Papers are not flexible, strong, smooth, non-shiny, durable, and white.
   - ☐ Papers are not typewritten or mechanically printed in permanent ink on one side.
   - ☐ Papers contain improper margins. Each sheet must have a left margin of at least 2.5 cm (1") and top, bottom and right margins of at least 2.0 cm (3/4").
   - ☐ Papers contain hand lettering.

2. Drawings are not in compliance and were not scanned because:
   - ☐ The drawings or copy of drawings are not suitable for electronic reproduction.
   - ☐ All drawings sheets are not the same size. Pages must be either A4 (21 cm x 29.7 cm) or 8-1/2" x 11".
   - ☐ Each sheet must include a top and left margin of at least 2.5 cm (1"), a right margin at least 1.5 cm (9/16") and a bottom margin of at least 1.0 cm (3/8").

3. Page(s) ______________ are not of sufficient clarity, contrast and quality for electronic reproduction.

4. Page(s) ______________ are missing.

5. OTHER: No Drawings